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Endogenous activation of GLP-1 receptor contributes to blood pressure control: role of proximal tubule NHE3, renal angiotensin II and insulin sensitivity

Authors: Flavia L. Martins, MSc*; Matthew A. Bailey, Ph.D.†; Adriana C. C. Girardi, Ph.D.*

Affiliations: *Heart Institute (InCor), Medical School, University of São Paulo, São Paulo, Brazil; †Centre for Cardiovascular Science, Queen's Medical Research Institute, The University of Edinburgh, Edinburgh, United Kingdom.

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Address correspondence to:

Adriana C. C. Girardi, Ph.D.

Laboratory of Genetics and Molecular Cardiology

Heart Institute (InCor)

University of São Paulo Medical School

Avenida Dr. Enéas de Carvalho Aguiar, 44, 10º andar, Bloco II

05403-900 – São Paulo, SP – Brazil

Phone: 55-11-2661-5929

E-mail: adriana.girardi@incor.usp.br

ABSTRACT

The pharmacological administration of glucagon-like peptide-1 receptor (GLP-1R) agonists reduces blood pressure in type 2 diabetes and non-diabetic patients. This study tested the hypothesis that endogenous GLP-1R signaling influences the regulation of blood pressure. To this end, spontaneously hypertensive rats (SHR) and Wistar rats were treated with the GLP-1R antagonist exendin-9 (Ex9) or vehicle for four weeks. Rats receiving the GLP-1R agonist exenatide (Ex4) were used as an additional control. We found that blockade of baseline GLP-1R signaling by Ex9 increased systolic blood pressure (SBP) in both SHR and Wistar rats, compared to vehicle-treated animals, while Ex4 only reduced SBP in SHR. Higher SBP induced by Ex9 was accompanied by reduced lithium clearance and lower levels of Na⁺/H⁺ exchanger isoform 3 (NHE3) phosphorylation at the serine 552, indicative of increased proximal tubule sodium reabsorption. Additionally, urinary angiotensinogen (AGT) and renal cortical concentration of angiotensin II (Ang II) were enhanced by Ex9. Conversely, Ex4 decreased both urinary AGT and cortical Ang II, but exclusively in SHRs. Moreover, both SHR and Wistar rats treated with Ex9 displayed hyperinsulinemia as compared to vehicle-treated rats, whereas Ex4 reduced fasting insulin concentration in SHR. Collectively, these results suggest that endogenous GLP-1R signaling exerts a physiologically relevant effect on blood pressure control, which may be attributable in part to its tonic actions on the proximal tubule NHE3-mediated sodium reabsorption, intrarenal renin-angiotensin system and/or insulin sensitivity. The possible role of impaired GLP-1R signaling in the pathogenesis of hypertension warrants further investigation.

KEYWORDS: hypertension, incretin, renoprotection, kidney, sodium reabsorption

INTRODUCTION

Glucagon-like peptide-1 (GLP-1) is an incretin hormone produced through differential post-translational processing of the proglucagon protein by the enteroendocrine L cells¹. Under physiological conditions, GLP-1 increases insulin secretion and suppresses glucagon release in a glucose-dependent manner². GLP-1 also increases insulin synthesis³ and insulin sensitivity in peripheral tissues⁴. All of these metabolic effects underscore the use of GLP-1 as a pharmacological target for treating type 2 diabetes (T2D). However, since native GLP-1 is rapidly degraded by the serine peptidase dipeptidyl peptidase-4 (DPP4)⁵, DPP4 inhibitors, and GLP-1 receptor (GLP-1R) agonists that are resistant to DPP4 degradation emerged as preferred anti-T2D drugs.

The glycemic effects of GLP-1 are primarily mediated through binding to its selective G protein-coupled receptor GLP-1R and formation of cAMP via Gs signaling⁶. Activation of the GLP-1R also leads to extra-glycemic effects in numerous tissues, including the kidneys. GLP-1R agonists have been demonstrated to induce diuresis and natriuresis, at least in part through inhibition of Na⁺/H⁺ exchanger isoform 3 (NHE3) in the renal proximal tubule⁷. GLP-1R induced cAMP activation also confers renal benefits on the diseased kidney, including antialbuminuric, antioxidant, and anti-inflammatory effects⁸.

Recent clinical studies indicate that the administration of GLP-1R agonists reduces blood pressure levels in diabetic patients when compared to placebo⁹⁻¹¹. The antihypertensive effect of GLP-1R agonists has also been observed in a variety of non-diabetic models of hypertension, including Dahl salt-sensitive rats¹², in angiotensin II-induced hypertension^{13, 14} and spontaneously hypertensive rats (SHR)¹⁵. Collectively, the results from these pre-clinical studies suggest that the antihypertensive effects of GLP-1 are mediated by its action on the kidneys to

induce natriuresis, on the endothelial cells to promote vasodilation and on the brainstem catecholamine neurons to attenuate sympathetic nerve activity. GLP-1R agonists have been demonstrated to counteract the actions of angiotensin II on the kidney¹⁶, which may represent other potential antihypertensive and renoprotective mechanism of these therapeutic agents. Notably, a study conducted in healthy subjects demonstrates a negative correlation between postprandial GLP-1 levels and systolic blood pressure (SBP)¹⁷, suggesting that endogenous GLP-1 may have blood pressure-lowering properties. Interestingly, we have previously found that acute blockade of the GLP-1R with its peptide exendin-9 exerts anti-natriuretic and antidiuretic effects in Wistar rats¹⁸. These results provide novel evidence that GLP-1 is a physiologically relevant natriuretic factor that contributes to sodium balance. However, it remains to be established whether endogenous GLP-1 may play a role in blood pressure control, and if so, the mechanisms involved. Therefore, this study aimed to test the hypothesis that long-term GLP-1R blockade increases blood pressure in both normotensive and hypertensive rats. Besides, a combined strategy using the GLP-1R antagonist exendin-9 and the agonist exenatide was employed to gain insight into the molecular mechanisms underlying the blood pressure effects of GLP-1R signaling.

METHODS

The data that support the findings of this study are available from the corresponding author upon request.

An expanded Material and Methods section is available in the online-only Data Supplement.

Animals - All experiments were carried out following the ethical principles in animal research of the Brazilian College of Animal Experimentation and were approved by the Institutional Animal Care and Use Committee. Forty-day-old male spontaneously hypertensive rats (SHR, $n = 54$) and Wistar rats ($n = 48$) were randomized into three groups and treated twice-daily with intraperitoneal injections of the GLP-1R antagonist exendin-9 (75 $\mu\text{g/kg/day}$), the GLP-1R agonist exenatide (10 $\mu\text{g/kg/day}$) or vehicle (0.9% saline) over 28 days. SBP was measured by tail-plethysmography at the start of the study (day 0) and thereafter weekly, commencing after treatment with exendin-9, exenatide, or saline. Five days before the end of treatment, rats were individually placed into metabolic cages for 24h urine collection. The diuretic and natriuretic responses to an acute extracellular volume expansion were evaluated three days before the end of the study. Oral Glucose Tolerance Test (OGTT) was performed two days before the end of treatment. On the last day of treatment, BP was measured by cannulation of the carotid artery in anesthetized rats. Arterial blood was collected at the time of death. Rats were killed by decapitation, and the kidneys were immediately removed for isolation of renal cortical membrane proteins and immunoblotting or tissue fixation for immunohistochemistry (Figure S1 and Table S1).

Statistical analysis - Results are reported as means \pm SEM with n indicating the number of rats unless otherwise stated. Comparisons among the groups were performed using one-way or

two-way ANOVA with repeated measures followed by the Bonferroni post-hoc test. $P < 0.05$ was considered significant.

RESULTS

Long-term blockade of GLP-1R increases blood pressure in both hypertensive and normotensive rats

Tail cuff BP was similar among the three groups of 40-day-old SHR_s before treatment (Figure 1A). In vehicle-treated rats, tail-cuff BP increased significantly over the 4-week experimental time course, consistent with developing hypertension in SHR_s. In rats receiving exendin-9, the hypertension was amplified compared to vehicle (Figure 1A), and at the end of the experiment, tail-cuff BP was 10 ± 2 mmHg higher than control. In contrast, the GLP-1R agonist exenatide attenuated the progressive rise in tail-cuff BP (Figure 1A), so that after 4 weeks was 12 ± 1 mmHg lower than in untreated animals. Surprisingly, exendin-9 treatment also increased tail-cuff BP by 16 ± 2 mmHg in conscious Wistar rats, but in this case BP was not reduced by activation of GLP-1R by exenatide (Figure 1B).

Next, we examined the effects of long-term blockade of GLP-1R on SBP and DBP in anesthetized SHR_s and Wistar rats. In SHR_s, we again found contrasting effects of exendin-9, which increased SBP compared to controls, and exenatide, which lowered SBP (Figure 1C). Normotensive Wistar rats treated with the GLP-1R antagonist exendin-9 also exhibited increased SBP compared to vehicle controls, whereas receptor agonism was without effect (Figure 1D). No significant differences were found in DBP among the three groups of SHR_s (Figure 1E). DBP was also similar across all three groups of Wistar rats (Figure 1F). Heart rate was not different across treatment groups in either SHR or Wistar (data not shown).

Long-term GLP-1R blockade impairs the acute renal natriuretic response ~~extracellular fluid volume homeostasis~~ and enhances proximal tubule sodium reabsorption of SHR_s and Wistar rats

Urine flow rate, glomerular filtration rate (GFR) and the fractional excretion of sodium, examined by 24h urine collection at the end of the study, were similar and unchanging in all groups (Tables S2 and S3). This is consistent with steady state sodium-fluid balance since food and water intake were not different between groups. However, long-term blockade or activation of the GLP-1R altered the acute (3h) excretory response to an intraperitoneal bolus of isotonic saline (10% volume/body weight). Chronic exendin-9 treatment reduced the percentage of the fluid and sodium load that was excreted in both SHR and Wistar rats (Figure 2A-D). Conversely, the acute diuretic/natriuretic response of exenatide-treated SHR was higher than that of controls and exendin-9-treated SHRs (Figure 2A and 2C). Similar fluid and salt load percentages were excreted by control and exenatide-treated Wistar rats (Figure 2B and 2D).

To evaluate whether changes at the rate of which the saline load was excreted by SHR and Wistar rats were accompanied by changes in proximal tubule sodium reabsorption, we measured the lithium clearance, an index of proximal tubular fluid delivery. As depicted in Figure 2, lithium clearance was decreased by exendin-9 and increased by exenatide when compared to SHR (Figure 2E) or Wistar rats (Figure 2F) that received vehicle.

Long-term blockade of GLP-1R reduces PKA-mediated NHE3 phosphorylation in the renal cortex of SHRs and Wistar rats

NHE3 is the primary pathway for sodium reabsorption in the proximal tubule apical membrane¹⁹. Therefore, we hypothesized that blockade of the GLP-1R signaling might be accompanied by stimulation of NHE3. To this end, we evaluated whether exendin-9 could alter NHE3 phosphorylation at the PKA consensus site serine 552 (PS552-NHE3) as a surrogate of

NHE3 activity since the levels of NHE3 phosphorylation at this residue negatively correlates with proximal tubule NHE3 transport function^{20, 21}. As illustrated in Figure 3A, long-term treatment with exendin-9 reduced the levels of PS552-NHE3 in the renal cortex of SHR, whereas exenatide modestly increased it. A similar reduction in the levels of PS552-NHE3 was observed in the renal cortex of Wistar rats treated with exendin-9. In line with earlier studies evaluating the acute effect of exenatide on NHE3⁷, long-term activation of the GLP-1R increased PS552-NHE3 levels in Wistar rats (Figure 3B). Conversely, total NHE3 protein abundance did not vary in response to either exendin-9 or exenatide treatments (Figure S2).

Long-term blockade of GLP-1R increases renal cortical angiotensin II (Ang II) concentration in SHR and Wistar rats

An activated intrarenal renin-angiotensin system (RAS) plays a crucial role in the pathogenesis of hypertension. We therefore evaluated the potential relationship between blockade of GLP-1R signaling and activation of the intrarenal renin-angiotensin system. We first assessed whether long-term blockade of GLP-1R could alter the urinary excretion of angiotensinogen (AGT), which is known to provide a specific index of intrarenal RAS status²². As illustrated in Figure 4, exendin-9 treatment enhanced urinary excretion of AGT in both SHR (Figure 4A) and Wistar rats (Figure 4B) as compared to their respective controls (vehicle-treated rats). Exenatide treatment reduced urinary AGT excretion, but only in SHR (Figure 4A-B). The systemic levels of AGT, evaluated by immunoblotting, remained unchanged in response to either exendin-9 or exenatide in both strains of rats (Figure S3).

Next, to confirm the effect of GLP-1R blockade on intrarenal RAS, the levels of renal cortical Ang II concentration was measured. Indeed, renal cortical Ang II content was increased

by exendin-9 in both SHR^s (Figure 4C) and Wistar rats (Figure 4D). Once again, exenatide treatment significantly reduced renal Ang II content in SHR^s (Figure 4C) but did not alter it in Wistar rats when compared to saline-treated animals.

Long term-blockade of GLP-1R leads to fasting hyperinsulinemia in normotensive rats, potentiates it in hypertensive rats and impairs insulin sensitivity in both rat strains

Hyperinsulinemia results from an imbalance between insulin secretion and insulin sensitivity²³. The association of hypertension, insulin resistance, and resultant hyperinsulinemia is well established²⁴. Given the strong evidence suggesting that GLP-1R activation increases insulin sensitivity in peripheral tissues^{25, 26} and thereby reduces fasting hyperinsulinemia²⁶, we tested the hypothesis that the effects of chronic blockade of GLP-1R on blood pressure could be associated with the worsening of insulin resistance.

The fasting blood glucose levels were similar among the SHR groups (Table S2). However, the OGTT demonstrated a significant increase in the area under the curve (AUC) of the SHR^s treated with exendin-9 as compared to vehicle-treated SHR^s (Figure 5A), suggesting that GLP-1R blockade induces some degree of glucose intolerance in SHR^s despite normal fasting glucose levels. Conversely, exenatide reduced AUC in SHR^s in comparison with controls (Figure 5A). In Wistar rats, fasting blood glucose levels and glucose tolerance were unaltered by treatment with exendin-9 (Table S3, Figure 5B); however, a reduction of AUC was observed in response to exenatide (Figure 5B).

As also illustrated in Figure 5, serum insulin levels were increased in exendin-9 treated SHR^s (Figure 5C) and Wistar rats (Figure 5D) compared to saline-treated controls. Notably, exenatide treatment reduced serum insulin levels of SHR rats to levels similar to those of saline-

treated Wistar. Insulin resistance was confirmed by calculating the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) index (Figure 5E-F). Long-term blockade of GLP-1R increased the HOMA-IR index in both SHRs and Wistar rats as compared to their respective saline-treated group. Activation of GLP-1R by exenatide improved insulin resistance in SHRs but did not affect it in Wistar rats (Figure 5E-F).

Long-term blockade of GLP-1R exacerbates renal damage in hypertensive rats

Recent clinical and experimental studies have highlighted the potential renoprotective actions of GLP-1R agonists in diabetic nephropathy that seem to be mediated by pleiotropic effects independent from glycemic control²⁷. Thus, we predicted that chronic blockade of the GLP-1R could potentiate the progression of renal damage, especially in SHRs. We found that four-week treatment with exendin-9 significantly raised the albumin to creatinine ratio (ACR) in SHRs and Wistar rats compared to the respective saline-treated control rats (Table S2-S3). Exenatide treatment decreased the ACR in hypertensive rats, but did not affect albuminuria in Wistar rats (Table S3). Figure S4 shows that the myofibroblast activation marker α -SMA was higher in SHR treated with exendin-9 than in those which received vehicle (Figure S4A) and that the number of infiltrating macrophages in the renal cortex was increased also compared to vehicle-treated rats (Figure S4B). Conversely, the renal cortex of SHRs treated with exenatide displayed lower expression of α -SMA (Figure S4A) and a reduced number of infiltrated macrophages (Figure S4B) when compared with saline-treated SHR rats. Neither expression of α -SMA nor infiltration of macrophages were detected in the renal cortex of Wistar rats treated or not with GLP-1R modulators (Figure S4C-D).

Systemic administration of GLP-1R antagonist and agonist alters GLP-1R signaling in the renal cortex

To determine whether the systemic infusion of exendin-9 or exenatide for four weeks affects GLP-1R signaling in the renal cortex of SHRs and Wistar rats, phosphorylation of PKA substrates were assessed by immunoblotting. As shown in Figure 6A, the levels of several phosphorylated PKA substrates were lower in the renal cortex of exendin-9-treated SHRs compared with SHRs that received saline. Conversely, the levels of phospho-PKA proteins were higher in the renal cortex of exenatide-treated vs. saline-treated SHR rats. As seen in Figure 6B, qualitatively similar effects of exendin-9 treatment were observed in Wistar, but the reduced phospho-PKA proteins were not statistically significant (78 ± 8 vs. $100 \pm 1\%$ Wistar-Ctrl, $P = 0.09$). However, exenatide administration was capable of increasing the phosphorylation levels of renal cortical PKA substrates in comparison with saline-treated Wistar.

DISCUSSION

Previous studies from our laboratory and others have demonstrated that acute pharmacological stimulation of the GLP-1R induces diuresis and natriuresis by increasing GFR and inhibiting the main renal proximal tubule sodium reabsorption pathway, NHE3, in normotensive rats^{7, 28, 29}. In addition, we have shown that acute blockade of the GLP-1R receptor provokes the opposite effect, i.e., decreases GFR and increases NHE3-mediated sodium reabsorption in the proximal tubule¹⁸, supporting the notion that endogenous GLP-1R signaling exerts a tonic natriuretic action that modulates sodium balance and may prevent volume expansion. Herein, we provide novel evidence that long-term blockade of the endogenous GLP-1R signaling by its antagonist exendin-9 intensifies blood pressure rise and exacerbates renal damage in hypertensive rats. Intriguingly, GLP-1R blockade also enhances blood pressure in normotensive rats, suggesting that endogenous GLP-1R signaling may contribute to physiological blood pressure homeostasis. Moreover, the results presented in this study suggest that the antihypertensive effects of baseline GLP-1R activation involve, at least in part, modulation on the proximal tubule NHE3-mediated sodium reabsorption, intrarenal renin-angiotensin system and/or insulin sensitivity.

Multiple clinical trials assessing the cardiovascular safety of the GLP-1R agonists have consistently shown that sustained treatment with these agents induces modest to significant reductions in systolic blood pressure of T2D hypertensive patients³⁰⁻³². DBP is less consistently affected. Notably, a Japanese study that included 128 subjects from the general population indicated that the postprandial increase of GLP-1 in response to a glucose load inversely correlates with SBP¹⁷. Conversely, no significant association was detected between postprandial GLP-1 levels and DBP¹⁷. These reports are in line with our findings that show that long-term blockade of GLP-1R signaling enhances SBP but does not affect DBP in normotensive and

hypertensive rats. The fact that SBP is the predominant component affected by GLP-1R signaling in human subjects as well as in our current experimental study suggests that GLP-1R-mediated blood pressure control is mainly due to its effects on extracellular volume homeostasis. This hypothesis is supported by the saline challenge experiments revealing that the blockade of GLP-1R by exendin-9 reduces the natriuretic/diuretic response to an acute extracellular volume expansion in both hypertensive and normotensive rats. Our data also indicate that rats treated with the GLP-1R antagonist exendin-9 ultimately maintain salt balance but a logical conclusion is that this comes at the expense of increased blood pressure.

The natriuretic/diuretic response is dependent on extracellular fluid volume and body sodium content (perhaps reference the paper mentioned by the reviewer here: PMID: 4021315). We observed a blunted excretory response to a saline load in exendin-9-treated rats, which points to underlying changes in extracellular fluid volume and may additionally reflect a decrease in GFR and/or an increase in tubular sodium reabsorption. In normotensive rats, the acute anti-natriuretic effect of exendin-9 is accompanied by a reduction in GFR and renal blood flow¹⁸. In contrast, these renal hemodynamic parameters are increased by acute GLP-1R activation, most likely due to the vasodilation of the afferent arterioles³³. However, chronic modulation of GLP-1R signaling does not significantly change GFR in normotensive rats and thus hemodynamic actions are unlikely to contribute significantly to the reduced natriuretic response to volume expansion in these animals. Notably, hypertensive rats are refractory to the acute GLP-1R-activation mediated renal vascular relaxation^{34,35}. In line with these findings, we show herein that long-term blockade or activation of the GLP-1R receptor does not affect GFR in hypertensive rats.

Proximal tubule NHE3-mediated sodium reabsorption plays a crucial role in the pressure natriuresis response that maintains volume homeostasis and blunts further increases in blood pressure³⁶. Our finding that higher blood pressure and impaired renal salt handling in hypertensive and normotensive rats treated with exendin-9 was associated with lower levels of NHE3-PS552 and higher lithium clearance suggests that long-term blockade of GLP-1R signaling elevates blood pressure at least in part by blunting the compensatory natriuretic response of the renal proximal tubule. In the kidneys, the GLP-1R is found only in the proximal tubule and glomerulus⁷. However, we cannot exclude the possibility that changes in renal GLP-1R signaling may also exert indirect effects on other sodium transporters along the nephron of hypertensive and normotensive rats, either due to the changes in the sodium load that is delivered to the distal nephron, or to the actions that GLP-1 has been shown to exert in opposition to those of Ang II¹⁶.

The RAS plays a determinant role in regulating blood pressure as well as sodium and water balance. Our study demonstrates that long-term blockade of GLP-1R activates intrarenal RAS. Thus, endogenous activation of the GLP-1R may prevent blood pressure elevation and volume expansion by downregulating intrarenal RAS. Indeed, several studies support the idea that GLP-1 is capable of counteracting the actions of systemic and renal Ang II^{13, 14, 16, 37}. Skov and colleagues have found that systemic infusion of GLP-1 in young healthy males for 2-hours decreases the circulatory Ang II concentration³⁷. The GLP-1R agonists exendin-4¹⁴ and liraglutide¹³ mitigate increases of blood pressure rise in response to Ang II, at least in part due to its natriuretic actions. A cross-talk between GLP-1R and Ang II has also been shown in isolated mesangial cells from human kidneys, in which GLP-1 blocked the Ang II-induced increase in reactive oxygen species and activation of inflammatory mediators through a GLP-1R/PKA

signaling pathway¹⁶. Opposing effects of GLP-1 and Ang II are also observed with regard to proximal tubule NHE3 regulation³⁸. While GLP-1 inhibits NHE3 by stimulating the cAMP/PKA signaling pathway and increasing the levels of NHE3-PS552^{7, 29}, Ang II reduces cAMP/PKA-mediated NHE3 phosphorylation at serine 552, leading to increased NHE3-mediated transport in renal proximal tubule cells³⁸. Accordingly, long-term administration of exendin-9 lowers cortical NHE3-PS552 levels. This effect might be resultant from the coordinated actions of the direct blockade of the renal GLP-1R signaling by this antagonist coupled with the indirect activation of the angiotensin II receptor type 1 (AT1R)/inhibitory Gi protein due to exendin-9-induced augmentation of cortical Ang II content. Under physiological conditions, the natriuretic effects of endogenous activation of GLP-1R signaling might neutralize the cellular pathway triggered by Ang II in the renal proximal tubule, thereby contributing to the maintenance of sodium balance and blood pressure regulation.

Insulin resistance and hyperinsulinemia aggravate hypertension, among other cardiovascular diseases^{24, 39}. In this context, our study shows that both hypertensive and normotensive rats treated with exendin-9 exhibit increased fasting serum insulin concentration and HOMA-IR index as compared to vehicle-treated rats. Indeed, our data indicate that long-term blockade of the GLP-1R is able to worsen insulin sensitivity in SHR and develop insulin resistance in Wistar rats. Hyperinsulinemia may occur as a compensatory mechanism of the pancreas to secrete higher amounts of insulin in the face of defects in the complex cascades of insulin receptor signaling, including insulin substrate 1/2 (IRS-1/2)/phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT). In the kidney, insulin resistance is selective⁴⁰ since IRS1 signaling is impaired⁴¹, whereas IRS2 remains intact^{42, 43}. The main effects arising from hyperstimulation of IRS2 in the kidneys are hyperfiltration and renal tubule-mediated sodium

retention^{41, 44}. Cumulative evidence indicates that hyperinsulinemia induces volume expansion and contributes to the development of hypertension, mainly by stimulating sodium reabsorption in the proximal tubule through NHE3 on the apical surface of the cell⁴⁵ and by the sodium/bicarbonate cotransporter (NBCe1) on the basolateral surface⁴¹. Thus, it is tempting to speculate that hyperinsulinemia contributes to blood pressure increase in our experimental models via proximal tubule NHE3. Nevertheless, despite the observations that higher blood pressure induced by long-term GLP-1R blockade is associated with NHE3 stimulation and increased insulin circulating levels, they are still not sufficient to validate this hypothesis.

Experimental and clinical evidence indicates that GLP-1R agonists, as well as DPP4 inhibitors, confer therapeutic benefits in diabetic nephropathy beyond its anti-hyperglycemic effects²⁷. These incretin-based agents confer renoprotection by reducing proteinuria, increasing GFR, and improving renal damage. The mechanisms underlying the renoprotective effects of GLP-1R agonists involve a reduction in oxidative stress, apoptosis signaling, inflammation, and protection from endothelial dysfunction. In line with the renoprotective actions of GLP-1R agonists, the present study reveals that long-term blockade of GLP-1R in SHR, a hypertensive model not associated with hyperglycemia, increases albuminuria, renal fibrosis, and inflammation. An important caveat is that we cannot discern from our results whether GLP-1R blockade worsens renal damage because it increases blood pressure or if inhibition of renal GLP-1R signaling *per se* may promote albuminuria, fibrosis, and inflammation. Interestingly, an in vitro study conducted in mesangial cells demonstrated that GLP-1 completely blocked the angiotensin II-induced generation of reactive oxygen species, NF- κ B activation, up-regulation of mRNA levels of intercellular adhesion molecule-1 and plasminogen activator inhibitor-1¹⁶.

These antioxidant and anti-inflammatory actions of GLP-1 were dependent on PKA activation¹⁶ and suggest that GLP-1R signaling may indeed be directly responsible for the renoprotection.

PERSPECTIVES

Evidence from clinical trials supports that GLP-1R agonists are able to reduce cardiovascular events and all-cause mortality as well as the progression of kidney disease in T2D. Apart from lowering glycemia, the cardioprotective benefits of the GLP-1R agonists may be attributable, at least in part, by their antihypertensive action. To the best of our knowledge, this is the first study to demonstrate that endogenous GLP-1R signaling exerts a physiologically relevant effect on blood pressure control. As such, GLP-1 may not only be a target for therapeutic intervention, but likely may contribute to the development and/or maintenance of hypertension. Further studies are warranted to elucidate the potential role of impaired renal GLP-1R signaling in the pathogenesis of hypertension.

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DISCLOSURES

The authors declare no conflict of interest.

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NOVELTY AND SIGNIFICANCE

1) What Is New?

- Several studies have consistently reported the diuretic, natriuretic, and antihypertensive effects of pharmacological GLP-1R activation. However, whether signaling through GLP-1R influences blood pressure at baseline has never been investigated previously.
- This is the first study that tested the hypothesis that endogenous GLP-1R signaling contributes to blood pressure control and to shed light upon its underlying mechanisms.

2) What Is Relevant?

- GLP-1R signaling at baseline contributes to blood pressure control and exerts tonic actions on proximal tubule NHE3-mediated sodium reabsorption, intrarenal RAS, and on insulin sensitivity.
- Impaired GLP-1R signaling may contribute to the pathogenesis of hypertension, among other cardiovascular, renal, and metabolic diseases.

3) Summary

In this study, we administered the GLP-1R antagonist exendin-9 to both hypertensive and normotensive rats in an attempt to validate the hypothesis that endogenous GLP-1R signaling plays a role in blood pressure control. Our results provide novel evidence that endogenous GLP-1R signaling regulates systolic blood pressure, which may be attributable, at least in part, to its tonic actions on the proximal tubule NHE3-mediated sodium reabsorption, intrarenal renin-angiotensin system and/or insulin sensitivity.

FIGURE LEGENDS

Figure 1 – Effects of long-term blockade and activation of GLP-1R on blood pressure of SHR and Wistar rats. (A-B) Tail-cuff blood pressure was measured before and weekly after drug treatment in (A) SHR (n = 18 rats/group) and (B) Wistar rats (n = 16 rats/group). (C-H) Before euthanasia, rats were submitted to cannulation of the carotid artery for direct measurement of (C-D) SBP and (E-F) DBP of SHR and Wistar rats. The values represent individual measurements and the means \pm SEM. *P<0.05 **P<0.01 ***P<0.001 vs. Ctrl and †††P<0.001 vs. Ex9.

Figure 2 – Effects of long-term blockade and activation of GLP-1R on extracellular volume homeostasis and lithium clearance in SHR and Wistar rats. A-D: SHR and Wistar rats treated with saline (Ctrl), exendin-9 (Ex9) or exenatide (Ex4) were challenged with an intraperitoneal bolus of warmed saline equivalent of 10% of their body weight and placed into metabolic cages for 3-h urine collection. (A-B) The percentage of the fluid load that was excreted within 3 hours of the saline challenge in SHR (A) and Wistar rats (B). (C-D) The percentage of the sodium load that was excreted within 3 hours of the saline challenge in SHR and Wistar rats. (E-F) Lithium clearance of SHR and Wistar rats. The values represent individual measurements and the means \pm SEM. *P<0.05 **P<0.01 vs. Ctrl and †††P<0.001 vs. Ex9.

Figure 3 - Effects of long-term blockade and activation of GLP-1R on the phosphorylation levels of NHE3 in the renal cortex of SHR and Wistar rats. Levels of phosphorylated (PS552-NHE3) and total NHE3 were determined by immunoblotting in the renal cortex of (A) SHR and (B) Wistar rats treated with saline (Ctrl), exendin-9 (Ex9) or exenatide (Ex4). Graphical

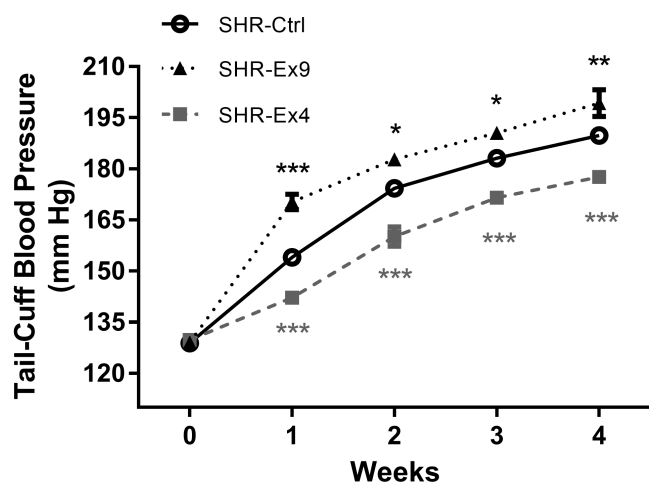
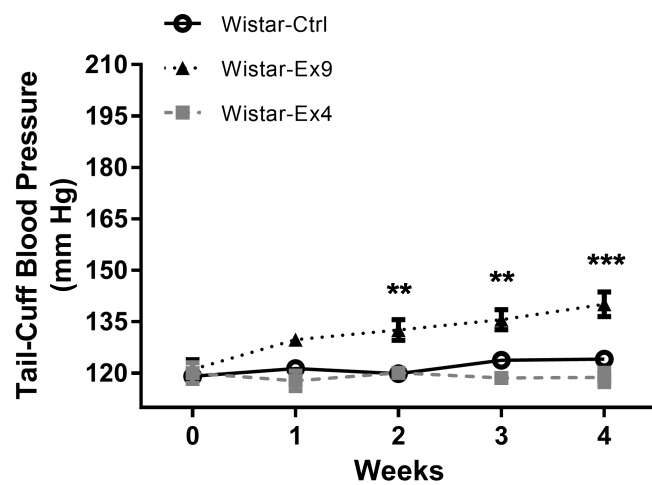
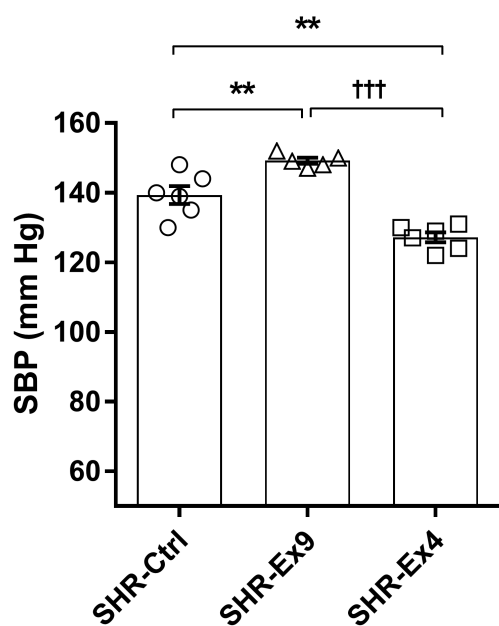
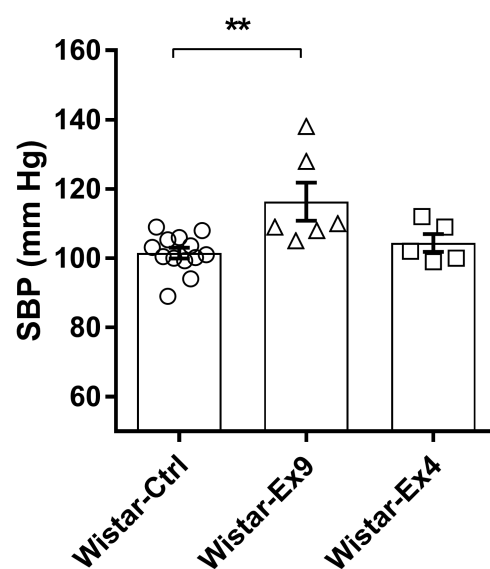
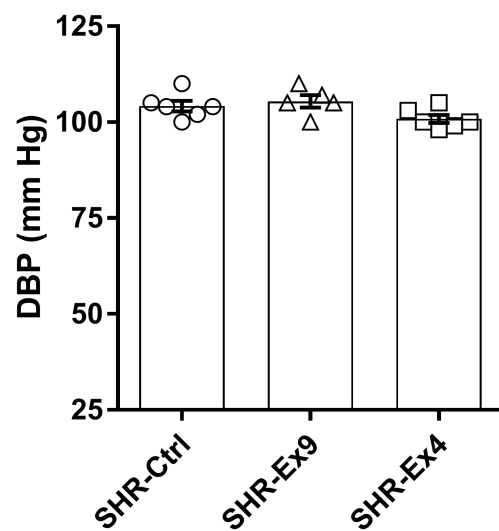
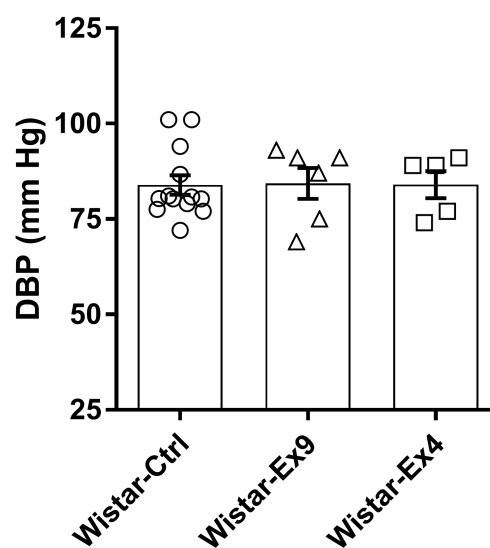
representation of the ratio of phosphorylated NHE3 to total NHE3. The values represent individual measurements and the means \pm SEM. * $P < 0.05$ ** $P < 0.01$ vs. Ctrl and ††† $P < 0.001$ vs. Ex9.

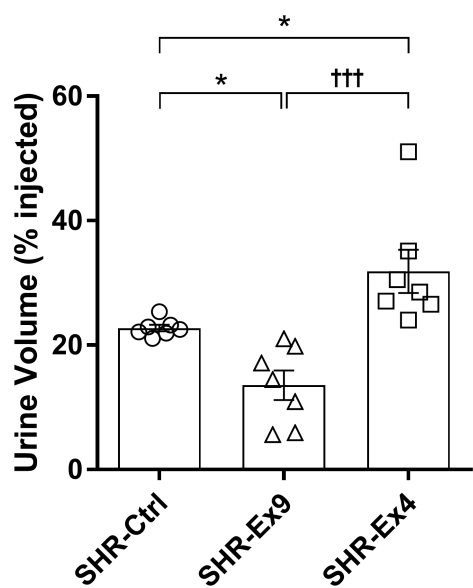
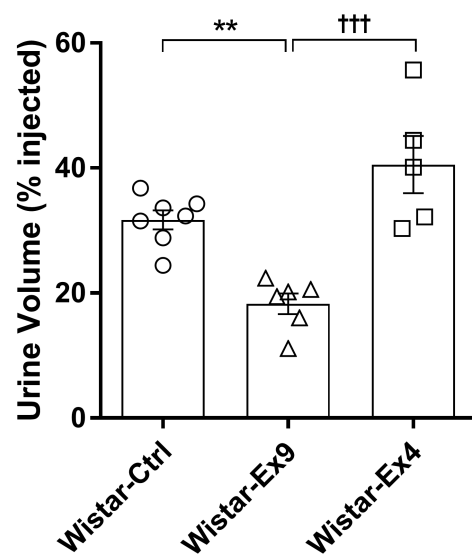
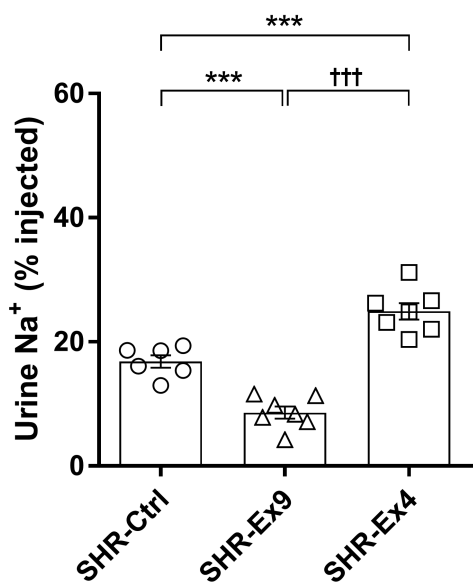
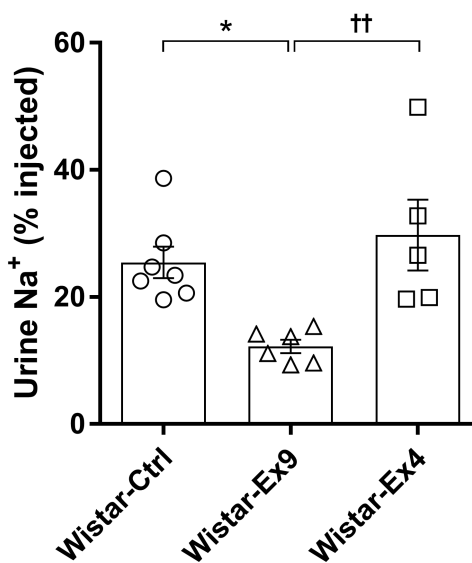
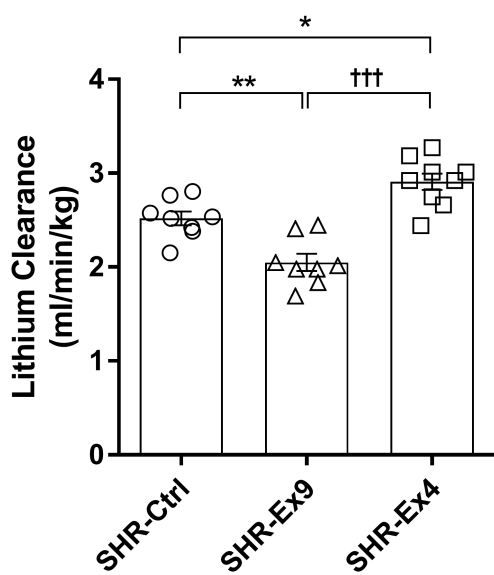
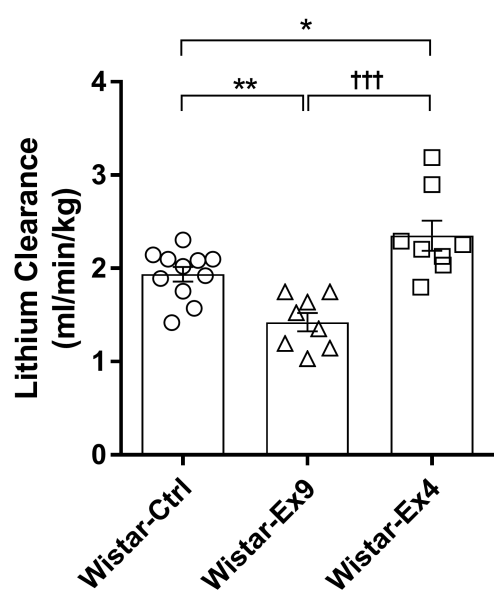
Figure 4 – Effects of long-term blockade and activation of GLP-1R on components of the intrarenal RAS. (A-B) The urinary angiotensinogen to creatinine ratio in **(A)** SHR and **(B)** Wistar rats treated with vehicle (Ctrl), exendin-9 (Ex9) or exenatide (Ex4). **(C-D)** The renal cortical angiotensin II (Ang II) content in **(C)** SHR and **(D)** Wistar rats. The values represent individual measurements and the means \pm SEM. * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ vs. Ctrl and †† $P < 0.01$ ††† $P < 0.001$ vs. Ex9.

Figure 5 – Effects of long-term blockade and activation of GLP-1R on glucose tolerance and insulin resistance in SHR and Wistar rats. (A-B) Oral glucose tolerance tests were performed following an 8-hour fast on **(A)** SHR and **(B)** Wistar rats treated with vehicle (Ctrl), exendin-9 (Ex9) or exenatide (Ex4) after an oral administration of glucose (2 g/kg of body weight). Glucose area under the curve (AUC) values were calculated. **(C-D)** Fasting serum insulin concentration in drug or vehicle-treated **(C)** SHR and **(D)** Wistar rats. **(E-F)** HOMA-IR index was calculated from fasting serum glucose and fasting serum insulin in **(E)** SHR and **(F)** Wistar rats. The values represent individual measurements and the means \pm SEM. * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ vs. Ctrl and † $P < 0.05$ †† $P < 0.01$ ††† $P < 0.001$ vs. Ex9.

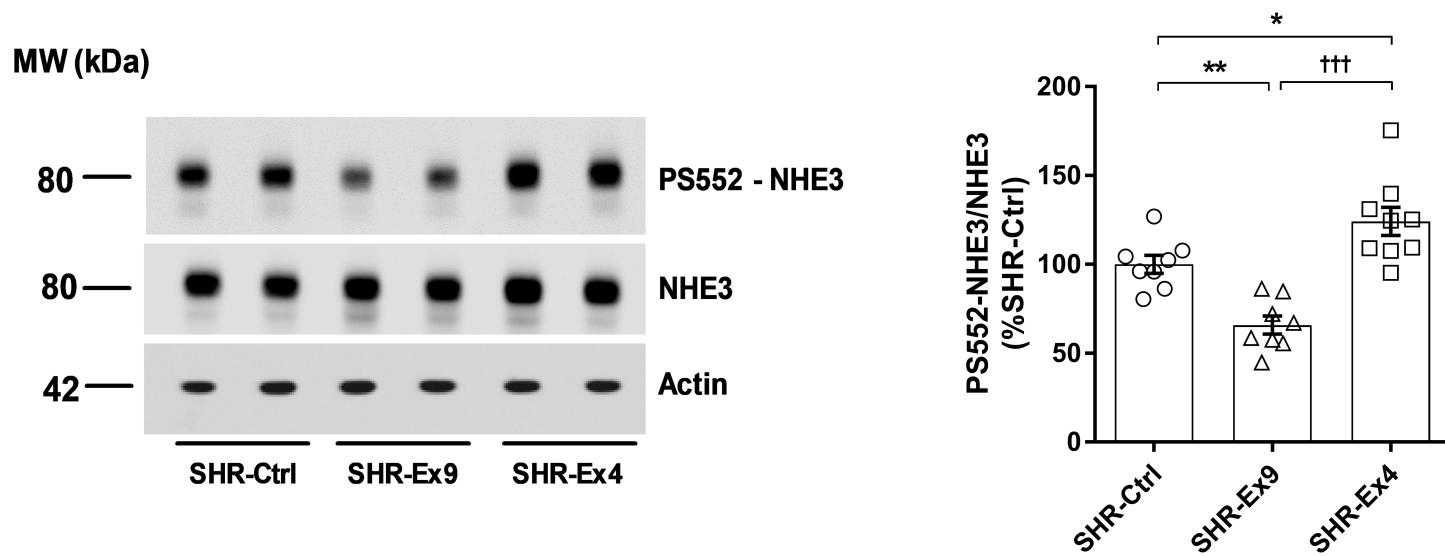
Figure 6 – Systemic treatment with the GLP-1R antagonist exendin-9 or the agonist exenatide affects renal cortical PKA activity. (A-B) Representative immunoblotting and

quantitation by densitometry of all phospho-PKA proteins in the renal cortex of **(A)** SHR and **(B)** Wistar rats treated with saline (Ctrl), exendin-9 (Ex9) or exenatide (Ex4). The values represent individual measurements and the means \pm SEM. *P<0.05 **P<0.01 vs. Ctrl and †††P<0.001 vs. Ex9.

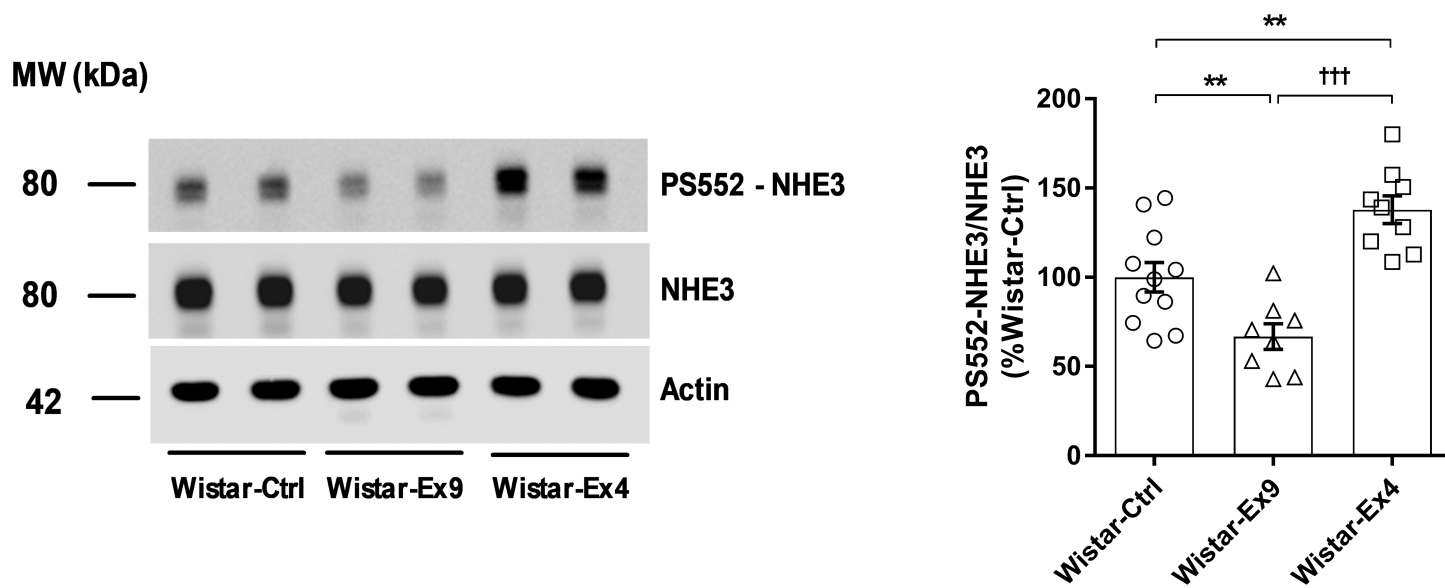
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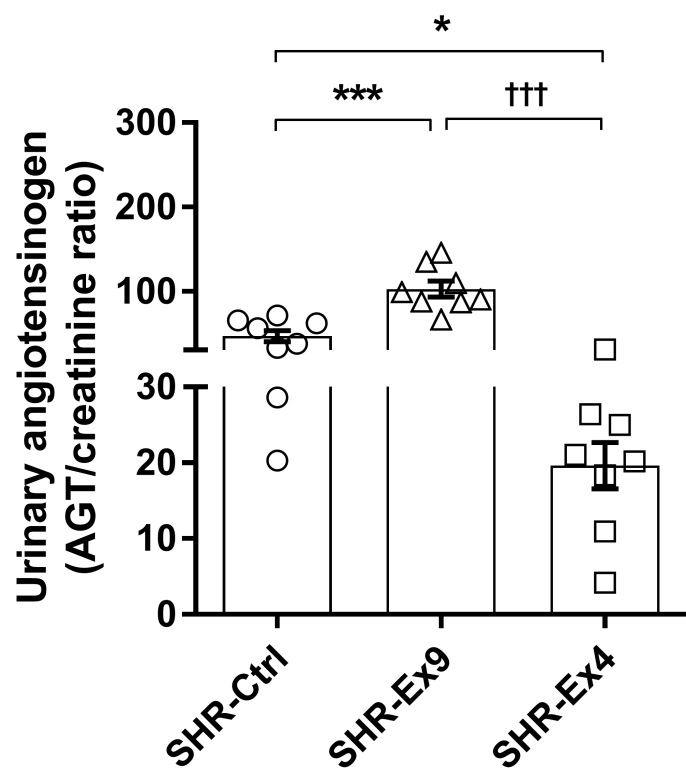
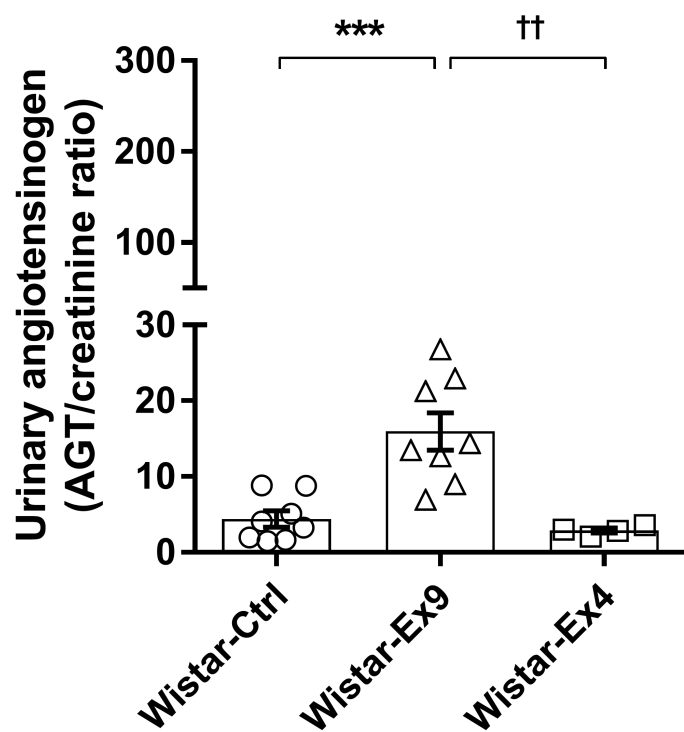
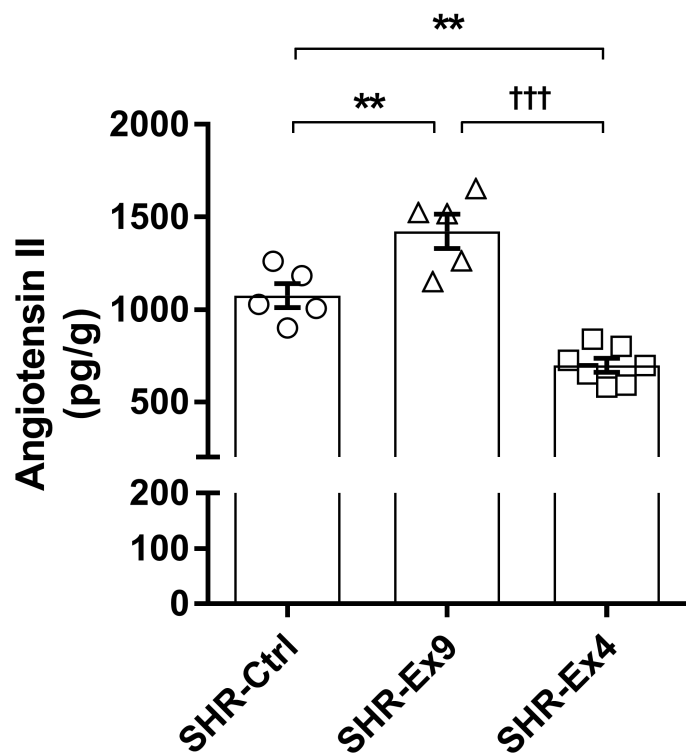
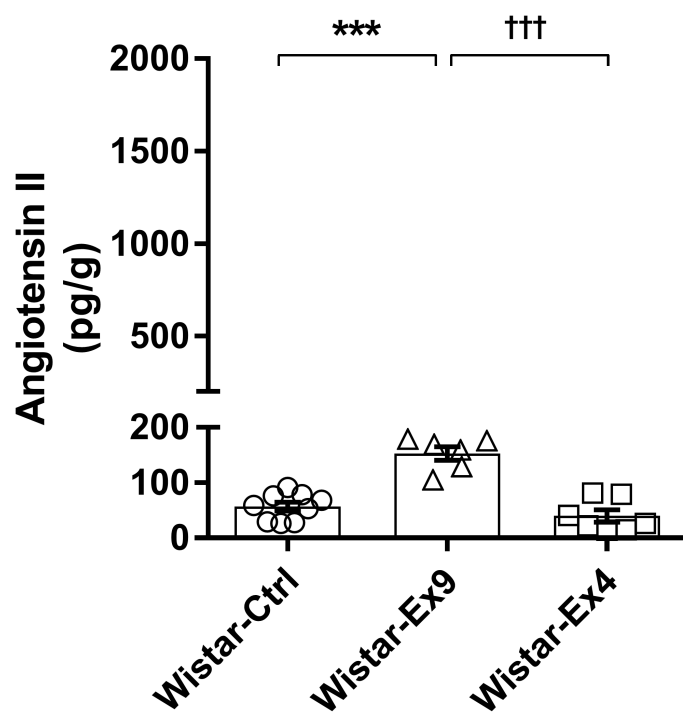
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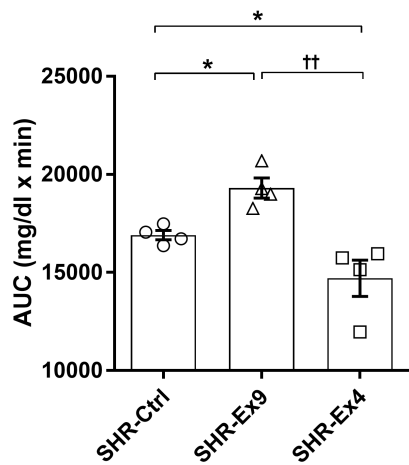
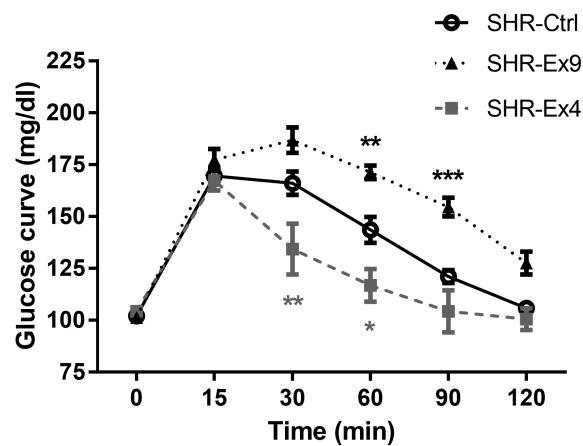
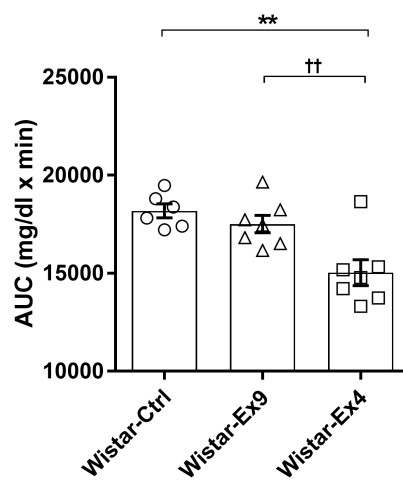
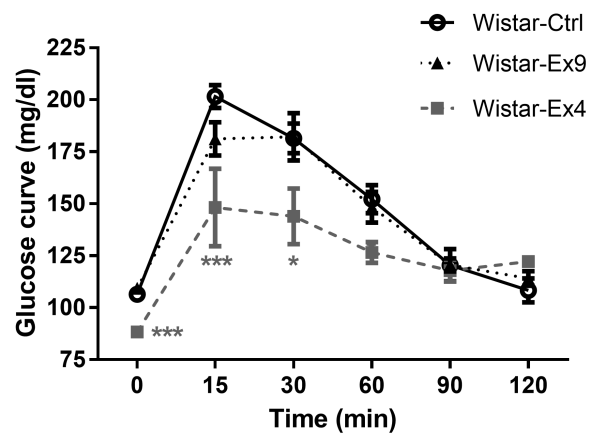
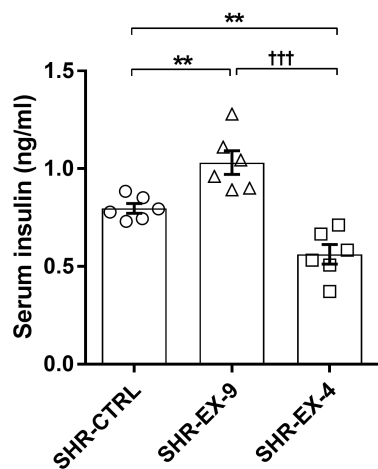
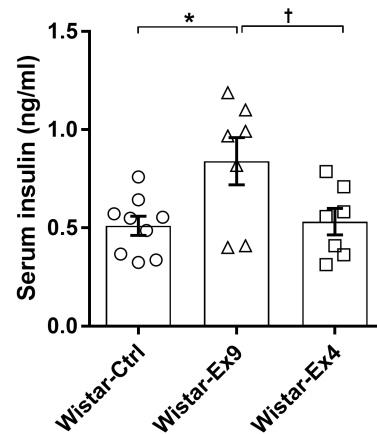
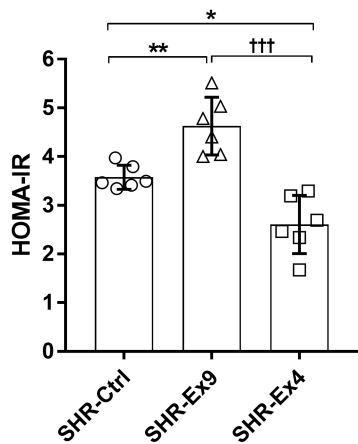
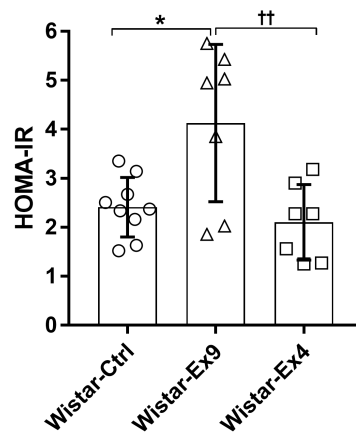
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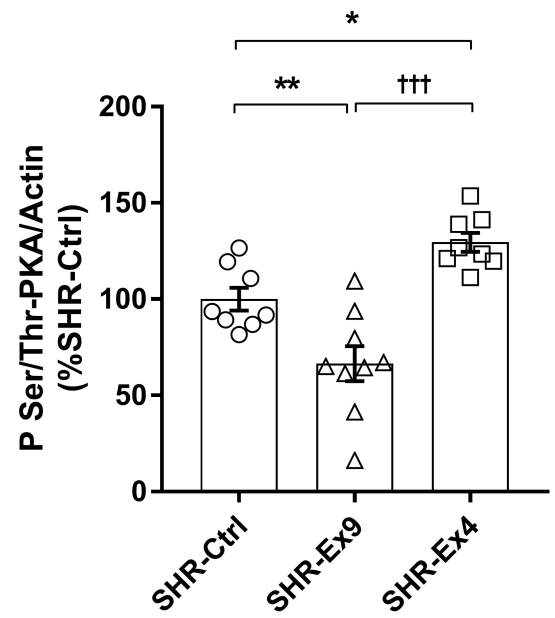
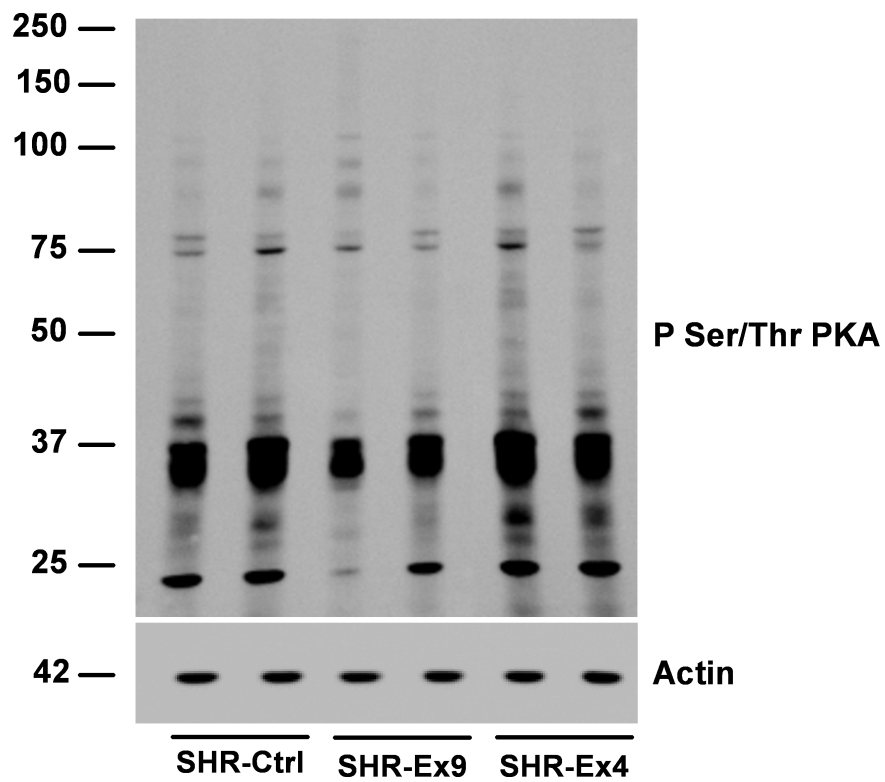


A**B****C****D**

A**B****C****D****E****F**

A

MW (kDa)

**B**

MW (kDa)

